SURFACE MODIFICATION FOR BIOCOMPATIBILITY

6th Quarterly Report
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PROJECT SUMMARY

The aim of this work is to create surfaces on implantable silicon microstructures for the purpose of controlling the interaction of neurons, glia, and related cells and their protein products with the microstructure. The third and fourth quarters were primarily devoted to the examination of culture conditions for early embryonic cultures with an aim to promote longevity in culture. We established E16 cortical cultures which survived to 21 days. E19 to 19 days. As presented in earlier reports, we established a rudimentary serum-free culture to approximate the composition of cerebral spinal fluid (CSF) and have extended screened artificial surfaces to: two (2) surfaces for E 14 response; nine (9) surfaces for E 16 cortical cell response, thirteen (13) surfaces for E19 cortical cell response; six (6) surfaces for E22 cortical cell response; two (2) surfaces for post-natal day 10 response; five (5) surfaces for glial astrocyte cell response, and three (3) surfaces for microglial response.

Last quarter we primarily focused on later embryonic (E22) cultures; and post-natal (PN10) cultures in which we initiated a preliminary screening of substrates and examined procedures most suited to establishment of long-term survival of these more challenging preparations. Experiments for culture of E14 embryonic tissue and post-natal day 2 microglia were also initiated.

In the sixth quarter we concentrated more fully on E 14 , astrocyte and microglial cultures, extending days in culture for astrocytes and microglial to 26 and 18 days, respectively. We examined the impact of substrate on neuronal subpopulations and neurotransmitter production (E14). We also concentrated on statistical analysis of the results of various experimental parameters (astrocyte), in addition to screening another substrate in the microglial culture.

Last quarter we showed improved stability of an artificial surface (silane monolayer on glass) in the presence of protein, but also identified that the glass we used, which is porous, dissolved after four to eight weeks -a complication which confuses the interpretation of the results. We have redesigned the initial experiment to include SiO_x, as well as glass, and investigated a new SAM, 13F-Cl_3.

Additionally, we have prepared various SAMs, with particular emphasis on modification with crosslinkers to allow attachment of biological macromolecules.

OBJECTIVES

Overall project objectives:

a) Selecting candidate surfaces that are likely to enhance the microscopic mechanical stabilization of a microstructure implanted within the central nervous system;

b) Selecting candidate organic surfaces that are likely to enhance the close approximation of neurons or neuronal processes to specific regions of implanted silicon microstructures;

c) Developing or adapting available methods to bond the selected organic molecules to a silicon dioxide surface like the surface of a micromachined electrode (Tanghe and Wise, 1992) and to chemically characterize these surfaces before and after protein adsorption.

   1. The attachment method shall be stable in saline at 37°C for at least 3 months;
   2. To use silane coupling as the method of attachment;
   3. To use the silanes to control the spatial extent (i.e., the pattern) of the deposited surface.

d) Developing a cell culture or other suitable model of mammalian cortex and investigate the growth and adhesion of neurons, glia, micro-glia, and other cells present in the nervous system on substrates coated with the selected surfaces;

e) Cooperating with other investigators in the Neural Prosthesis Program by coating microelectrodes (estimated 50 over the contract period) with the most promising materials for *in vivo* evaluation as directed by the NINDS Project Officer.

QUARTER OBJECTIVES

- Continue work to establish cortical cell culture conditions for optimal 4 week survival
- Begin screening biologically modified SAM surfaces for cortical cell survival
- Continue screening E14 response to artificial surfaces
- Finish screening E22 response to artificial surface
- Continue screening PN10 response to artificial surfaces
- Continue surface analysis of surfaces both before and after culture
- Continue surface stability experiments in saline
- Continue screening surfaces for microglia response
- Send samples to Huntington to continue *in vivo* experiments as well as explore other possibilities for collaboration in this area

BACKGROUND

Biomaterials that penetrate into the central nervous system as the microscopic electrode shafts of neural prostheses interact with neural and other tissues on a cellular and molecular level. In order to achieve tight coupling between these implanted microelectrodes and the target neural substrate, this interaction must be understood and controlled. Controlling the interaction requires an understanding of how cells, including neurons and glia, and extracellular proteins respond to the surface chemistry and any leachable substances of implanted biomaterials. This contract supported research will study these interactions with a long-term goal of rationally designing microelectrode surfaces to promote specific tissue interactions.

Presently, available clinical neural prosthetic implants typically use stimulus levels that excite volumes of neural tissue ranging from cubic millimeters to cubic centimeters around the electrode. Because of the large stimulus intensities required, precise control of the response of neurons within the first few cell layers of an implanted electrode has not been necessary. Recent developments in the areas of micromachining and fabrication of silicon integrated circuit microelectrodes have introduced the possibility of controlled stimulation of smaller

volumes of neural tissue--on the order of one thousand to one hundred thousand times smaller than those used today.

The efficiency of these microelectrodes depends on the micro-environment around stimulating sites. The surface of the microelectrodes and the proteins that adsorb to this surface have a major impact on the way in which different cell populations react to the implant. Neural growth cones are sent out from many neurons around a microelectrode following implantation. With appropriate surfaces it may be possible to get selected neurons to send processes directly to the microelectrodes. Glia and other cells also respond to an implanted electrode. With appropriate surfaces it may be possible to promote cell adhesion and anchoring of some areas of the implant structure while leaving other areas with minimal response from glial cells. This study will investigate cellular and molecular responses to specific surface modifications of silicon microelectrodes.

RESULTS

Surface Analysis and Stability Measurements

X-ray photoelectron spectroscopy (XPS) is necessary for this program in the same way that an NMR spectrometer is necessary for conducting an organic synthesis program. Since we are synthesizing surfaces and modifying their properties, we will need to assay the result of the surface before (starting material) and after (reaction product) modification. This is analogous to examining a procedure by NMR. One would not think to run a multistep organic synthetic reaction without an examination of the product at the end of each step; in the same sense, it is crucial for us to examine the product in our surface modification experiments.

The stability of the silane monolayers has been investigated in a new experiment in which two forms of the "13F" monolayer [R-CH_2-CH_2-$(CF_2)_5$-CF_3, R = Si-$(CH_3)_2$-Cl, or Si-Cl_3] have been tested on two different substrates, glass 22 X 22 mm cover slips (as in earlier

experiments, see quarterly reports 3 through 5) and on silicon wafers. The earlier tests showed improved stability in the presence of dissolved protein (0.001% BSA) but the interpretation was clouded by the dissolution of the glass cover slip substrates. In the current experiment which includes a higher concentration of BSA (0.1%), interestingly the trichloro 13F monolayer (on SiO_x) has been found to be more stable than the monochloro 13F for short periods.

We have also used XPS to analyze the amounts of protein deposited by cells onto the substrates in one microglia, and one astrocyte experiment. These post-mortem XPS results will be discussed in more detail in the next quarterly report.

Surface modification

In this quarter, we have concentrated on research and production of some novel SAMs, with particular emphasis on modification with biological macromolecules. One unique property of SAMs is the availability of surface functional groups which provide sites to do additional chemistry. As SAMs also provide a means of reproducibly controlling surface functionality, modification of the surface functional groups with crosslinkers will allow repeatable attachment of selected biological macromolecules. Crosslinking chemisty (both homofunctional and heterofunctional cross linkers) can be used to derivatize a SAM and provide a very selective and reactive surface to covalently link a number of different macromolecules to the surface. Several of these cross linkers have been explored for reaction to the functional head group of the SAM. A homobifunctional cross linker such as 1,4 phenylene diisothiocyanate can be reacted under basic conditions with these amine functionalities leaving one reactive isothiocyanate on the surface for reaction to any terminal amine from a biological protein such as BSA or laminin. This chemistry has been successfully applied to our surfaces and cell culture experiments are being initiated.

Cell Culture

As illustrated in previous reports, we have developed of a rudimentary serum-free culture system for the cortical cells to more closely approximate the composition of cerebral spinal fluid (CSF). In the fourth and fifth quarters, we concentrated on defining and examining the culture conditions to optimize longevity for both cortical and astrocyte cultures, in addition to initating microglial and post-natal day 10 cultures and extending substrate screening of E 22 and astrocyte cultures.

In the sixth quarter we concentrated on E 14 cultures, astrocyte and microglial cultures, extending both days in culture for astrocytes and microglial (26 and 18 days, respectively), and our understanding of cell-substrate interactions. In particular, we examined the effect of substrate and growth factors on neuronal subpopulations and neurotransmitter production (E14) and the effect of substrate and growth factors on survival, attachment, leading edge extension, branching, node formation (astrocytes) (to be presented more fully next quarter). In addition to performing XPS and substantial statistical analyses related to the E14 and astrocyte cultures, we examined the effect of substrate on adhesion/survival of microglial cultures (Figure 1).

Figure 1 shows a microglial culture at Day 12 fixed and stained with OX-42 (1:100, Serotech). Microglia plated on poly-D-lysine controls and DETA have improved attachment and survival as compared to cells plated on the 13F monolayer. The cultured microglia project thin processes and resemble ramified microglia.

Collaborations

We have established a collaboration with W. Agnew at the Huntington Medical Research Institute. While we have not yet specifically modified electrodes, we have successfully modified the poly-silastic sheath that holds a series of microelectrodes in place along the spinal cord during implantation (Agnew et al., 1990). One problem we are focused on solving is that the sheath has glial scar buildup and adhesions that eventually displace the electrodes laterally (with adhesion) and vertically (due to buildup of tissue). We visited

Huntington Institute after the Neuroscience meeting in November, and have planned a series of experiments. We will begin a new series of experiments once the costs of the *in vivo* work is determined. We are also examining by surface analysis some postmortem sample supplied by D. Agnew's group. These results are ongoing and will be reported at a later date.

NEXT QUARTER OBJECTIVES

- Continue work to establish cortical cell culture conditions for optimal 4 week survival
- Begin screening biologically modified SAM surfaces for cortical cell survival
- Continue screening E14 response to artificial surfaces
- Continue screening PN10 response to artificial surfaces
- Continue surface analysis of surfaces both before and after culture
- Continue surface stability experiments in saline on SiOX/glass
- Continue screening surfaces for microglia response
- Send samples to Huntington for *in vivo* experiments as well as explore other possibilities for collaboration in this area.

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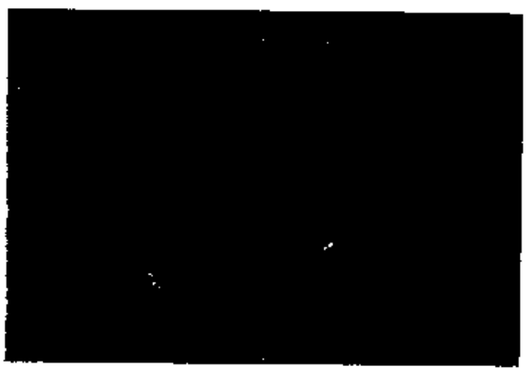
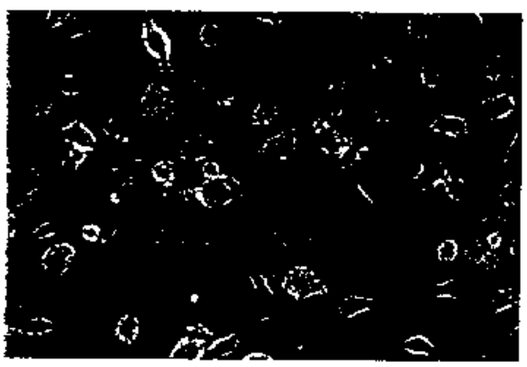
EFFECT OF SURFACES ON CORTICAL MICROGLIAL SURVIVAL AND GROWTH IN CULTURE

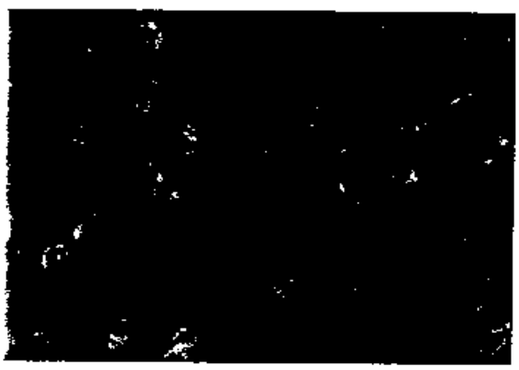
Phase-contrast

Anti-OX42

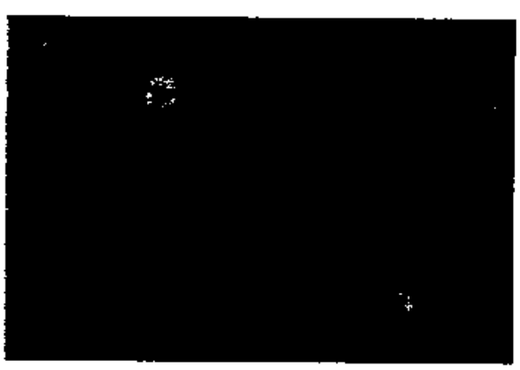


Poly-D-Lysine





DETA



13F

Enriched cultures of rat microglia derived from cerebral cortex